TRP Channels II

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Functional Expression of TRPC6 and TRPV4 Channels in Mouse Skeletal Muscle Fibers

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We have recently shown that the cation channels TRPC6 and TRPV4 are expressed in mouse skeletal muscle and are at least partially localized in the sarcolemma. However, little is known about their functions in skeletal muscle. To investigate the role of TRPC6 and V4 for calcium influx into muscle fibers we applied agonists and blockers of the above mentioned channels. ML-9, known as a myosin light chain kinase inhibitor, is also a potent blocker of TRPC6, but does not affect other TRP channels. 4α-PDD is known as TRPV4 activator and HC-067047 was shown to be a specific TRPV4 inhibitor. To investigate divalent cation influx we used single interosseus muscle fibers and applied the $Mn2^{+}$ quench technique using Fura-2. Quench of Fura-2 fluorescence was measured in response to excitation at 360 nm in the presence of 0.5 mM $Mn2^{+}$. OAG increased background calcium influx nearly twofold (control vs. OAG; 3.3 ±0.3 vs. 6.5±0.6 %/min; n=33; $p<0.01$), supporting the view of functional expression of TRPC3 and/or C6 in skeletal muscle fibers. This response was attenuated in the presence of 100 µM of ML-9 (OAG vs. OAG and ML-9; 4.0 ±0.3 vs. 3.5±0.6 %/min; n=36; not significant). ML-9 application to muscle fibers did not affect background calcium influx. Application of the TRPV4 activator 4α-PDD (5 µM) caused an increase in background calcium influx (control vs. 4α-PDD; 6.5 ±0.7 vs. 11.0±1.4 %/min; n=33; $p<0.01$). This increased calcium influx could be inhibited by 1 µM HC-067047. We conclude that both channels, TRPC6 and TRPV4 are functional in the sarcolemma of isolated mouse muscle fibers. Both channels do not seem to have any resting activity but they can be pharmacologically activated and blocked.

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Heteromeric TRPC3 with TRPC1 Formed via its Ankyrin Repeats Regulates the Resting Cytosolic Ca^{2+} Levels in Skeletal Muscle

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The main tasks of skeletal muscle are muscle contraction and relaxation, which are mediated by changes in cytosolic Ca^{2+} levels. Canonical-type transient receptor potential 3 (TRPC3) contains an ankyrin repeat (AR) region at the N-terminus (38-188 amino acids) and forms extracellular Ca^{2+}-entry channels by homo or heteromerization with other TRP subtypes in various cells including skeletal myotubes. However, previous research has not determined which region(s) of TRPC3 is responsible for the heteromerization, whether the AR region participates in the heteromerizations, or what is the role of heteromeric TRPC3s in skeletal muscle. In the present study, the heteromerization of TRPC3 with TRPC1 was first examined by GST pull-down assays of TRPC3 portions with TRPC1. The portion containing the AR region of TRPC3 was bound to the TRPC1, but the binding was inhibited by the very end sub-region of the TRPC3 (1-37 amino acids). In-silico studies have suggested that the very end sub-region possibly induces a structural change in the AR region. Second, the very end sub-region of TRPC3 was expressed in mouse primary skeletal myotubes, resulting in a dominant-negative inhibition of heteromeric TRPC3/1 formation. In addition, the skeletal myotubes expressing the very end sub-region showed a decrease in resting cytosolic Ca^{2+} levels. These results suggest that the AR region of TRPC3 could mediate the heteromeric TRPC3/1 formation, and the heteromeric TRPC3/1 could participate in regulating the resting cytosolic Ca^{2+} levels in skeletal muscle.

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Identification of an Essential Structural Element of Lipid Gating Mechanism in the Transient Receptor Potential Canonical Channel Type 3 (TRPC3)

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Transient receptor potential canonical 3 (TRPC3), is highly expressed in neuronal and cardiac tissues, and its importance in development and (patho) physiology of these tissues is well established. So far, our understanding of structure-function relations, specifically in terms of the lipid sensing machinery in this channel protein is incomplete.

Using a homology model of TRPC3, based on the recently available structural information on TRPV1, we performed structure-guided mutagenesis and identified a single residue within the transmembrane domain 6 (G652) as pivotal for lipid-mediated gating of TRPC3. Increasing the residue size in position 652 eliminated lipid sensitivity. $TRPC3_{G652A}$ expressed in HEK293 cells was found resistant to activation via the phospholipase C pathway or to direct administration of diacylglycerols. On the contrary, a synthetic agonist of TRPC3/6/7 channels (GSK1702934A) activated both wild-type and $TRPC3_{G652A}$ channels, generating even larger membrane conductances in the lipid-insensitive mutant. To gain further insight into the mechanisms of TRPC3 activation by GSK1702934A and diacylglycerols, we synthetized and characterized a series of structurally related analogs of GSK1702934A. Our results identify two groups of TRPC3 activators, one of which includes the putative physiological activator diacylglycerol and is largely inactive in $TRPC3_{G652A}$ channels.

We suggest that lipid gating of TRPC3 strictly requires a hinge-point or a certain level of flexibility within transmembrane segment S6 provided by G652. Lipids and synthetic activators of TRPC3 may be capable of initiating divergent gating movements in the channel.

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Ca^{2+} and Calmodulin Regulation in Receptor-Operated Cation Currents of TRPC6 Channels

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Calmodulin (CaM) contributes a variety of ion channels gating regulation in response to Ca^{2+} changes. However, it is still missing the molecular basis of CaM regulation in mammalian TRP channels which contribute to receptor-operated cation (Ca^{2+} and Na^{+}) currents. To accumulate of Ca^{2+} and CaM roles in TRP channels, we have firstly characterized the Ca^{2+} regulation in TRPC6 channel, which is highly expressed in pulmonary and vascular smooth muscle cells. The decay of the receptor-operated cation currents of TRPC6 channels was clearly delayed to EGTA or BAPTA chelation, which suggested the global Ca^{2+} mechanism. We then examined CaM binding to the C-terminal region of TRPC6 channels by using of Ca^{2+}-dependent FRET system. FRET due to CaM binding to the C-terminal region of TRPC6 channels demonstrated a bell-shape response curve to Ca^{2+} increments, which was a unique pattern compared to the IQ-domain of voltage-gated Ca and Na channels. And the bell-shape response curve was altered to a simple grow curve by a mutation in either N- or C-globular Ca^{2+}-binding domain (lobe) of CaM. Intriguingly, the mutant in the N-globular domain of CaM delayed the decay of receptor-operated currents of TRPC6 channels, thus the lobe-specific function of CaM appeared in TRP channels. These results indicated that the Ca^{2+}-dependent inactivation of TRPC6 channels can be explain by the bell-shape response curve of CaM binding which curve is probably caused by a conflictive binding between the N- and C-globular domain of CaM to TRPC6 channels. Our results provide a unique molecular basis of CaM to terminate ion channel activity which has critical roles in the down-stream of vasoconstrictors, transmitters and growth factors.

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An Alternative Ion Permeation Pathway in the TRPM3α1 Isoform?

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TRPM3 is a cation conducting ion channel that belongs to the superfamily of transient receptor potential (TRP) channels. It is polymodally regulated by stimuli including heat, hypoosmolarity, the endogenous agonist pregnenolone sulphate (PS) as well as the synthetic agonist nifedipine. Recently, we have shown that combined application of PS and the antifungal drug clotrimazole (Clt) leads to the activation of TRPM3 by inducing the opening of two pores, the central pore and an alternative ion permeation pathway. We have further identified a selective activator of TRPM3, CIM0216, which is able to open both pathways independently of the presence of other chemicals. At the moment a tremendous amount of TRPM3 splice variants have been described. In this study, we have selected one splice variant TRPM3α1, that differs from the TRPM3α2 isoform only in the presumed pore region. Remarkably, the TRPM3α1 isoform did not show any increase in channel activity